Accumulation of Nucleopolyhedrosis Virus of the European Pine Sawfly (Hymenoptera: Diprionidae) as a Function of Larval Weight[1]

M. A. MOHAMED, H. C. COPPEL, J. D. PODGWAITE,[2] AND W. D. ROLLINSON[2]
Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT Disease-free larvae of *Neodiprion sertifer* (Geoffroy) treated with its nucleopolyhedrosis virus in the field and under laboratory conditions showed a high correlation between virus accumulation and body weight. Simple linear regression models were found to fit viral accumulation versus body weight under either circumstance.

Much research and development toward the practical utilization of the nucleopolyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Geoffroy) has taken place both in North America and Europe. Although registration of this virus for commercial use in North America is imminent, its production remains limited to in vivo culture (Cunningham and Entwistle 1981). Given this limitation, optimization of all variables that will maximize production are necessary. Such variables include initial inoculum, larval weight or instar, and the density of larvae on a given food source. This paper explores the latter two variables in relation to viral accumulation in *N. sertifer* larvae. Further, since mortality response to fixed or varying doses of insect viruses are dependent on larval weight or stage (Hedlund and Yendol 1974, Burges and Thompson 1971), one would expect that viral accumulation would also be some function of larval weight or instar.

Materials and Methods

Two red pine, *Pinus resinosa* Ait., plots (0.04 ha each), in the southern district of the Kettle Moraine Forest of Wisconsin, were sprayed with the nucleopolyhedrosis virus (NPV) of *N. sertifer*. The virus was applied at 3.5×10^9 polyhedral inclusion bodies (PIB) per ha in a formulation in which each liter contained 4.6×10^8 PIB, 47 ml of Chevron Spray Sticker, 60 g of IMC-90-001 Shade, and 953 ml of water. Each plot contained trees arranged to hold either 5 or 10 colonies of sawfly larvae per tree. The spray was applied when the larvae were in the 2nd instar.

Five tagged trees in each density situation were monitored daily for 3 weeks. Each day, when available, a single dead larva was chosen at random from each colony per tree per density situation. The larvae were stored singly in sterile disposable tubes at 0°C. From the dead larval samples obtained, 40 and 34 larvae were subsampled at random to represent the two density situations of 5 and 10 colonies per tree, respectively.

To complement these field studies, laboratory experiments were conducted in a somewhat similar manner. That is, eggs collected from the same area were allowed to hatch. Individual colonies were placed on pine seedlings and then sprayed with an aqueous suspension of *N. sertifer* PIB when the larvae were in the 1st instar. The concentration used was 1×10^6 PIB/ml, each seedling being treated with 2 ml. From the larvae that died subsequently 18, 20, 41, and 38 were chosen at random to represent 1st, 2nd, 3rd, and 4th instars respectively.

Each larva sampled from either field or laboratory experiments, was weighed, its instar recorded, and it was then macerated in a glass tissue homogenizer. The numbers of PIB were counted with a Levy counting chamber and brightfield microscopy at 600×.

PIB accumulation as a function of body weight and instar was analyzed by correlation and linear regression.

Results and Discussion

The correlation between PIB accumulation and body weight from field-collected larvae was high (Table 1) in both density situations. Linear regression of PIB accumulation versus body weight (Fig. 1 and 2, Table 1) resulted in virtually similar, moderately high, R^2 values in both density situations. Residual analysis showed no evidence of trends or variance heterogeneity, indicating that a linear model is an appropriate fit for the data. Thus, it can be concluded that NPV accumulation is a linear function of body weight.

An attempt to fit a single regression line on the data from the two density situations resulted in a reduction of the R^2 value to 49.1%. Comparison of the two lines

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[2]Northeastern For. Exp. Stn., Hamden, CT 06514.

Table 1.—Linear regression equations and statistics of PIB accumulation (Y) in *N. sertifer* larvae as a function of body weight (X1)

Density situation (larval colonies/tree)	Regression equations[a]	R^2 (%)	Correlation coefficient[b]
5	$Y = 9.7 \times 10^4 + 2.6 \times 10^4 \times 1$	69	0.83
10	$Y = 3.1 \times 10^4 + 8.1 \times 10^4 \times 1$	66	0.81

[a]Slope terms of these equations significant ($P < 0.01$).
[b]Values significant at $P < 0.01$.

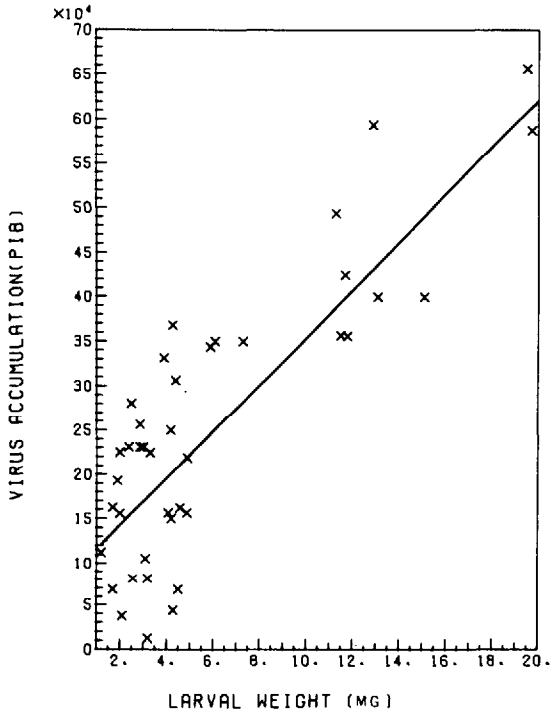

FIG. 1.—Regression line of virus accumulation versus body weight (five *N. sertifer* larval colonies per tree).

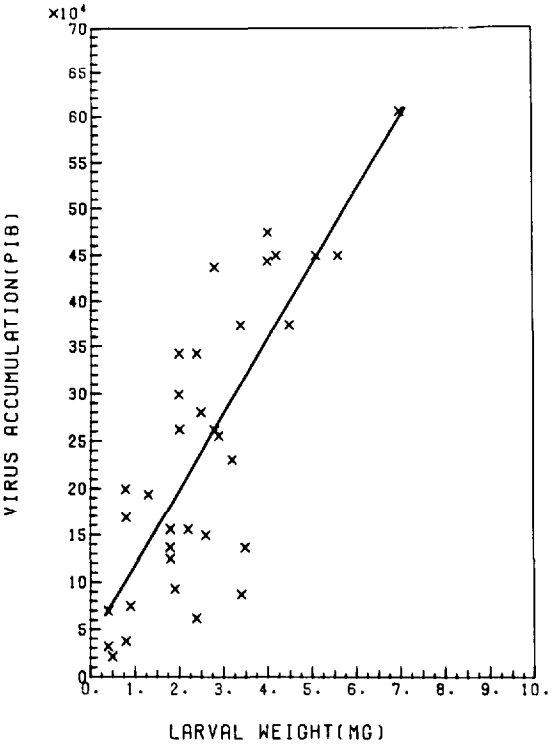

FIG. 2.—Regression line of virus accumulation versus body weight (10 *N. sertifer* larval colonies per tree).

at the two density situations resulted in $F(2, 70) = 61.0$, $P < 0.01$. These results possibly indicate that the density of larvae on a tree may affect the net accumulation of virus in larvae. The slope terms of the regression equations at the two density situations suggest that larvae in a high-density situation accumulate virus at a faster rate than those in a lower-density situation.

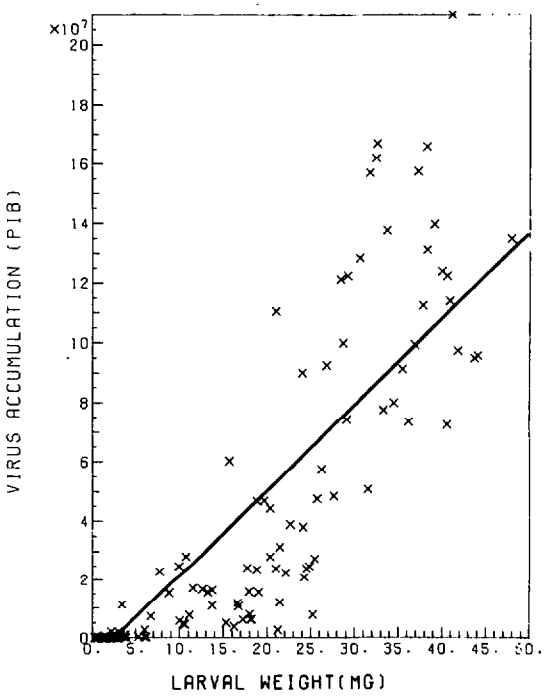

FIG. 3.—Regression line of *N. sertifer* virus accumulation versus body weight in laboratory studies.

Analysis of the data from the laboratory experiment (Fig. 3), resulted in a correlation of PIB accumulation and weight of 0.845 which was significant at $P < 0.01$. Linear regression on these data resulted in the equation $Y = -2.0 \times 10^7 + 3.0 \times 10^7$, where the R^2 value was 71.5%, and the slope term was significant at $P < 0.01$ (df = 115). There was no evidence of variance heterogeneity in residual plots, indicating that a linear model is correct for the data. These results compare favorably with those from field-collected larvae.

Multiple linear regression of PIB accumulation against both larval weight and instar gave no appreciable increase in the R^2 value for larvae either from the field or laboratory studies. Hence, the linear models with a single independent variable appear to be sufficient to explain the data.

The results of this study suggest that the in vivo production of *N. sertifer* virus may best be maximized by ensuring that larvae attain their maximum body weight either through adequate food supply or by creating a low density situation to mitigate any detrimental effects of intraspecific competition.

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